THE ACTION OF THE SALIVARY SECRETION OF MOSQUITOS AND OF *GLOSSINA TACHINOIDES* ON HUMAN BLOOD

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Whilst conducting experiments with malaria sporozoites from the salivary glands of *Anopheles maculipennis*, we were much impressed by the extraordinary degree of agglutination of the erythrocytes which immediately occurred on mixing an emulsion of salivary gland with citrated human blood. On looking through the literature, we were surprised to find that apparently the only record of this phenomenon is that of Cornwall and Patton (1914), who state that the salivary secretion of *A. rossi* and *A. jamesi* produced strong and immediate agglutination of the red corpuscles of human blood and prevented the blood from clotting. Experiments were undertaken to investigate this subject and to contrast the action on human blood of the salivary secretion of female *A. maculipennis* with that of female *Culex pipiens, Theobaldia annulata, Stegomyia fasciata* and *Glossina tachinoides.*

In all experiments, the emulsion of salivary glands was obtained by breaking up as finely as possible with needles the entire salivary glands from a single insect in 0·035 c.c. of physiological saline solution, and the citrated blood was obtained by adding three volumes of human blood to one volume of a 1 per cent. solution of sodium citrate in physiological saline solution ; one volume of the salivary gland emulsion was then mixed with two volumes of the citrated blood solution and the mixture drawn up into a capillary tube of rather wide bore.

In the case of *A. maculipennis* complete and immediate agglutination of the erythrocytes was observed, whereas in the case of *Culex pipiens, Theobaldia annulata, Stegomyia fasciata* and *Glossina tachinoides*, respectively, no trace of agglutination of the erythrocytes was seen, even after standing an hour at either 37° C. or laboratory temperature. The experiments, which were repeated several times, were performed with blood from two normal individuals and gave constant results.

Observations were next made in order to obtain some idea of the concentration of the agglutinin in the salivary gland emulsion of *A. maculipennis*. For this purpose the original emulsion containing the complete salivary glands of one mosquito in 0·035 cc. of saline solution was diluted 4 times, 8 times, 16 times, 32 times, 64 times and 128 times, respectively, and as in the previous experiments one volume of the various dilutions mixed with two volumes of citrated blood. Complete and immediate agglutination of the red cells was observed up to a dilution of 32-fold; with the 64-fold dilution there was a considerable immediate agglutination which became complete after standing a little time, while in the case of the 128-fold dilution the amount of agglutination observed was but slight. These observations show clearly that haemagglutinin is present in the salivary glands of *A. maculipennis* in very great concentration.

The agglutination of red cells due to the salivary secretion of *A. maculipennis* takes place about equally well at 37° C. as at laboratory temperature of about 15° C.

Heating the emulsion of salivary gland of *A. maculipennis* for half an hour at 56° C. completely destroys the agglutinin. Further experiments showed that exposure to a temperature of 50° C. for half an hour also completely destroyed the agglutinin, whilst heating to 45° C. for a similar period, produced some slight diminution in its intensity. The haemagglutinin in the salivary secretion of *A. maculipennis* is hence very thermolabile and is destroyed at comparatively low temperatures. When a mixture of emulsion of salivary glands of *A. maculipennis* and blood—the red cells of which are thus completely agglutinated—is heated at 60° C. for two minutes, it was found, on discharging the blood on a glass slide, that the agglutinated masses of red corpuscles had completely dissociated

and no longer re-agglutinated when the temperature fell to 15° C. Heating the mixture to 50° C. for fifteen minutes partially destroyed the agglutinated masses, whilst heating at the same temperature for thirty minutes caused complete disappearance of the agglutination.

The haemagglutinin present in the salivary glands of *A. maculipennis* differs, therefore, in certain respects from the autoagglutinin present in the blood of certain normal animals, and more markedly in the blood of these animals when suffering from certain infections, e.g., trypanosomiasis : firstly, in that the salivary gland agglutinin acts about equally well at 37° C. as at low temperatures, and secondly, in that it is very thermolabile, being destroyed at 50° C.

If the salivary gland emulsion of *A. maculipennis* is allowed to dry at either 15° C. or 37° C. and the residue re-dissolved in a volume of fluid equal to the original volume of the emulsion, it is found that on adding two volumes of citrated blood no agglutination takes place : the agglutinin is thus destroyed by dessication.

Adopting the same technique, it was found that the salivary secretion of *A. maculipennis* agglutinated strongly, but not quite so intensely as in the case of man, the red blood corpuscles of the donkey, rabbit and dog, but had no action on those of the mouse, guinea-pig and monkey (*Cercopithecus* sp.). The salivary secretion of *Stegomyia fasciata* had no agglutinating action on the red corpuscles of any of these animals.

Emulsions of the stomach and of the ventral oesophageal diverticulum of *A. maculipennis* failed to produce any agglutination of human erythrocytes.

No evidence of haemolysis was observed in any of the experiments performed with the salivary gland emulsion of *A. maculipennis*, or any of the other mosquitos, or of *Glossina tachinoides*.

Experiments were finally undertaken with *A. maculipennis*, *Stegomyia fasciata* and *Glossina tachinoides* to determine whether the salivary gland emulsion exerted any inhibitory action on the coagulation of human blood. The salivary gland emulsions were made as described above, the complete glands of one insect being emulsified in 0·035 cc. of physiological saline. One volume of the emulsion was then mixed with two volumes of blood as it escaped from the freshly-punctured finger and after mixing thoroughly,

drawn up into a capillary tube of rather wide bore and placed in a water bath at 37° C. The control tubes containing one volume of physiological saline and two volumes of blood were invariably found to have clotted after five minutes, so that when the contents of the tube were blown out on to a glass slide, a long worm-like red clot was extruded : this red cylindrical mass could not be disintegrated by stirring with a needle or by warming to 60° C., as would be the case if the red cells were merely agglutinated. The salivary secretion of *Stegomyia fasciata* had no effect on the coagulation, the blood having clotted as in the controls within five minutes ; the salivary gland emulsion of *A. maculipennis* merely delayed the coagulation of the blood, no clot being observed after five minutes, but a distinct clot was found after fifteen minutes. In the case of *Glossina tachinoides*, however, the anticoagulating power of the salivary gland emulsion was much more pronounced, no coagulation of the blood being observed after standing at 37° C. for thirty minutes and little, if any, even after twelve hours.

In view of the above observations one is led to enquire what happens to the blood which is drawn into the mosquito stomach on feeding. It was found that the blood in the stomach of *A. maculipennis* two hours after feeding on a human being, exhibited complete agglutination of the erythrocytes, but was unclotted, whereas that in the stomach of *Stegomyia fasciata* a similar time after feeding, exhibited no agglutination of the erythrocytes, but was quite definitely clotted. This suggests that when the mosquito feeds, salivary secretion is first poured out into the wound and then partly withdrawn with the blood into the stomach. The fact that sporozoites were found free amongst the blood in the stomach of an infected *A. maculipennis* two hours after feeding, is additional evidence in support of this; as the mosquito had been infected over five weeks before the meal in question, the sporozoites found amongst the blood in the stomach could not have been contaminations from oöcysts in the stomach—and none were found in the stomach on dissection—but must apparently have had their origin in salivary secretion which, after being discharged into the wound, had been withdrawn with the blood into the stomach. Furthermore, a little blood is occasionally to be found in the ventral oesophageal diverticulum of an *A. maculipennis* when examined an hour or two after feeding

and sporozoites have been found amongst the blood in this situation, a couple of hours after feeding.

We can offer no explanation of the function of the haemagglutinin or anticoagulin in the salivary secretion of *A. maculipennis*. So far both these bodies have been shewn to be present in the salivary secretion of all the *Anopheles* examined, viz., *A. rossi*, *A. jamesi* and *A. maculipennis*, but both are absent in that of *Stegomyia fasciata*, whilst in *Glossina tachinoides* the salivary secretion contains a powerful anticoagulin, but no agglutinin.

SUMMARY

1. An emulsion of the salivary glands of *A. maculipennis* powerfully agglutinates the erythrocytes of human blood and, less strongly, those of the donkey, rabbit and dog ; it has no agglutinating action on the red corpuscles of the mouse, guinea-pig or *Cercopithecus* sp. Emulsions of the stomach and of the ventral oesophageal diverticulum exerted no agglutinating action.

2. The haemagglutinin is thermolabile being destroyed by heating to 60° C. for two minutes or to 50° C. for thirty minutes, and the agglutinated red cell masses dissociate completely under these conditions.

3. Similar salivary gland emulsions of *Culex pipiens*, *Theobaldia annulata*, *Stegomyia fasciata* and *Glossina tachinoides* do not agglutinate human red cells, nor does the salivary gland emulsion of *Stegomyia fasciata* agglutinate the red cells of any of the animals mentioned above.

4. No haemolysin was detected in the salivary gland emulsion of any of these insects.

5. The salivary gland emulsion of *Stegomyia fasciata* exerted no anticoagulating action on human blood, whilst in the case of *A. maculipennis* and of *Glossina tachinoides*, the anticoagulating action was quite definite.

6. The blood in the stomach of *A. maculipennis* two hours after feeding on a human being was found to exhibit complete agglutination of the erythrocytes, but to be unclotted, whilst that in the stomach of *S. fasciata*, a similar period after feeding on man, exhibited no agglutination, but was coagulated.

7. This indicates that the salivary secretion is in part withdrawn into the stomach with the blood when the mosquito feeds ; further evidence in support of this is the fact that sporozoites were found intermingled with the blood in the stomach and ventral oesophageal diverticulum of infected *A. maculipennis* examined shortly after feeding.

REFERENCE

Cornwall, J. W., and Patton, W. S. (1914). Some Observations on the Salivary Secretions of the Commoner Insects and Ticks. *Ind. Journ. Med. Res.* Vol. II, p. 569.

ADDENDUM

Since writing the above, we have had an opportunity of examining the reactions of the salivary secretion of *A. bifurcatus* and find that it neither causes agglutination of human erythrocytes nor does it delay coagulation of human blood. In these respects, therefore, *A. bifurcatus* differs strikingly from *A. maculipennis*, *A. rossi*, and *A. jamesi*.